Targeting Ara h 2 with human-derived monoclonal antibodies prevents peanut-induced anaphylaxis in mice

Paolucci, Marta ; Wuillemin, Natascha ; Homère, Valentine ; Bieli, Dimitri ; Köhli, Alice ; Ballmer-Weber, Barbara ; Waeckerle-Men, Ying ; Pengo, Niccolò ; Kündig, Thomas M ; Sonati, Tiziana ; Johansen, Pål

DOI: https://doi.org/10.1111/all.15659

Received: 3 October 2022 | Revised: 15 December 2022 | Accepted: 30 December 2022

DOI: 10.1111/all.15659

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

Targeting Ara h 2 with human-derived monoclonal antibodies prevents peanut-induced anaphylaxis in mice

Marta Paolucci[1] | Natascha Wuillemin[2] | Valentine Homère[1] | Dimitri Bieli[2] | Alice Köhli[3] | Barbara Ballmer-Weber[4] | Ying Waeckerle-Men[1] | Niccolò Pengo[2] | Thomas M. Kündig[1,4] | Tiziana Sonati[2] | Pål Johansen[1,4]

[1]Department of Dermatology, University of Zurich, Zurich, Switzerland

[2]Mabylon AG, Schlieren, Switzerland

[3]Division of Allergology, University Children's Hospital Zurich, Zurich, Switzerland

[4]Department of Dermatology, University Hospital Zurich, Zurich, Switzerland

Correspondence
Pål Johansen, Department of Dermatology, University of Zurich, Rämistrasse 100, 8091 Zurich, Switzerland.
Email: pal.johansen@usz.ch

Funding information
Innosuisse - Schweizerische Agentur für Innovationsförderung; Truus und Gerrit van Riemsdijk Stiftung; Universität Zürich; Swiss Government Excellence Scholarships for Foreign Scholars and Artists; University of Zurich

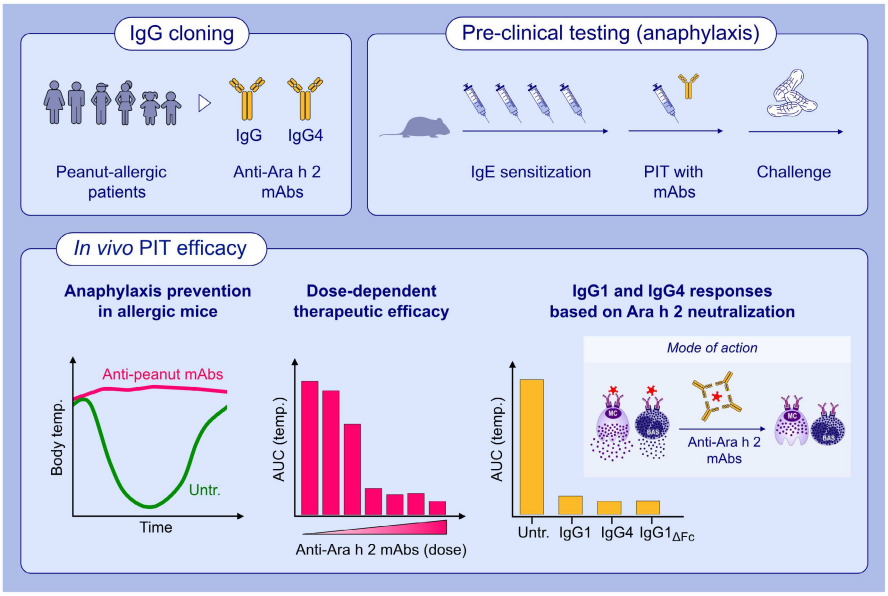
Abstract

Background: Peanut allergy is a type-I hypersensitivity immune reaction mediated by the binding of peanut allergens to IgE-FcεRI complexes on mast cells and basophils and by their subsequent cellular degranulation. Of all major peanut allergens, Ara h 2 is considered the most anaphylactic. With few options but allergen avoidance, effective treatment of allergic patients is needed. Passive immunotherapy (herein called PIT) based on prophylactic administration of peanut-specific monoclonal antibodies (mAbs) may present a promising treatment option for this under-served disease.

Method: Fully human recombinant anti-peanut IgG mAbs were tested in mice sensitized to peanut allergen extract. Allergic mice received intravenous immunotherapy with anti-peanut Ara h 2-specific IgG1 or IgG4 mAbs cocktails, and were then challenged by a systemic injection of high-dose peanut allergen extract. The protection from allergic anaphylaxis was measured by monitoring the core body temperature.

Results: PIT with peanut-specific mAbs was associated with a significant and dose-dependent reduction of anaphylactic reactions in peanut-sensitized mice challenged with peanut allergen extract. Complete protection was observed at doses approximately 0.3–0.6 mg mAbs. Mixtures of mAbs were more effective than single mAbs, and effective treatment could be obtained with mAbs of both IgG1 and IgG4 subclasses. The therapeutic effect of anti-Ara h 2 mAbs was based on allergen neutralization and independent of the Fcγ receptor and mast-cell inhibition.

Conclusion: This is the first report that shows that human-derived anti-peanut mAbs can prevent allergic anaphylaxis in mice. The study demonstrates that neutralizing allergenic epitopes on Ara h 2 by mAbs may represent a promising treatment option in peanut-allergy.



GRAPHICAL ABSTRACT

Anti-Ara h 2 IgG1/IgG4 mAbs from peanut-allergic patients were cloned and recombinantly produced. Preclinical testing was performed in peanut-sensitized mice receiving passive immunotherapy prior to an allergen challenge. Anti-Ara h 2 mAbs prevent allergic anaphylaxis by neutralizing peanut allergens before they bind to IgE and induce a FcεRI-mediated degranulation of mast cells and basophils.
Abbreviations: AUC, area under the curve; FcεRI, Fc epsilon receptor I; Ig, immunoglobulin; mAbs, monoclonal antibodies; PIT, passive immunotherapy; temp., temperature

1 | INTRODUCTION

Peanut-induced food allergy is a major health issue with increasing prevalence among children.[1–3] Allergic reactions include anaphylaxis and occur when allergen binds IgE-FcεRI complexes on the surface of mast cells and basophils. The resulting cross-linking of FcεRI receptors triggers cellular degranulation with release of mediators, example, histamine and sulfidoleukotrienes.[4] Seventeen peanut allergens have been described (www.allergen.org). Of these, Ara h 2 is immunodominant[5] and recognized by more than 90% of peanut-allergic patients.[6–8] Ara h 2 sensitization has been shown to be associated with systemic reactions including anaphylaxis.[9]

Management of peanut allergy is primarily based on dietary avoidance, but peanut storage proteins are typically heat stable and frequently contaminants of foods.[10] Even small amounts of peanuts can cause severe anaphylaxis.[11] Allergen immunotherapy (AIT) by subcutaneous administration of peanut allergen extract has been performed, but stopped due to high frequency of severe adverse events, including death.[12,13] Oral allergen immunotherapy (OIT) was recently approved for children and adolescents and shall induce immune tolerance by the administration of increasing doses of peanut proteins.[14] One mechanism of AIT involves the stimulation of allergen-specific IgG antibodies that neutralize allergen and block allergen binding to IgE.[15–17] In patients undergoing peanut OIT[18–20] or otherwise tolerant,[4,21,22] increased allergen-specific IgG4 and reduced IgE levels were reported, and increased IgG4-IgE ratios were observed for antibodies targeting a single Ara h 2 epitope.[19] Since oral tolerance induction to foods is mostly transient, continued OIT is required, and severe adverse reactions may be expected,[23] making new therapeutic approaches an urgent need.

Given the safety risks of peanut AIT, passive immunotherapy (PIT) by administration of peanut-specific IgG antibodies represents a promising treatment alternative for peanut-allergic patients. It is expected that antibodies can mediate immune protection via neutralization of the allergen, thereby preventing its IgE binding and subsequent triggering of mast cell and basophil degranulation.[24–26] In addition, IgG can potentially bind the inhibitory receptor FcγRIIb and inhibit the activation of effector cells.[24,27,28] So far, monoclonal antibodies (mAbs) specific for cat dander[25] or birch pollen[26,29,30] allergens are in clinical development. We currently develop mAbs for treatment of peanut allergy. The fully human mAbs are targeting Ara h 2, and for selection of most promising candidates, the current study tested the therapeutic efficacy of anti-peanut mAbs in mouse models of peanut-allergic anaphylaxis.

2 | MATERIALS AND METHODS

2.1 | Peanut-specific monoclonal antibodies

Molecular cloning of antibodies specific to peanut allergens is described in the supplemental materials (Appendix S1). Briefly, B cells from peanut-allergic patients were isolated and tested for anti-Ara h 2 antibodies. Single cells were isolated, and the variable regions were cloned into expression vectors containing IgG1, IgG4, and kappa or lambda human constant regions. Subsequently, antibodies

were produced recombinantly in HEK cells and purified via protein A affinity columns. Single peanut allergen-specific mAbs, recognizing different regions of Ara h 2, were named MY-mAb-1, MY-mAb-2, MY-mAb-3, and MY-mAb-4. Quadruple mixtures of the same mAbs were named MY-4-IgG1 or MY-4-IgG4, depending on the immunoglobulin subclass.

2.2 | Mice

Female C3H/HeNHsd (C3H), and BALB/cOlaHsd (BALB/c) mice were purchased from Envigo (Horst, NL) and used at age 6–10 weeks. All animals were kept under specific pathogen-free conditions at the Biologische Zentrallabor (BZL), University Hospital Zurich, and at the Laboratory Animal Services Center (LASC), University of Zurich, in individually ventilated cages, at 21°C, with a 12–12 h light–dark cycle, and with free access to water and chow. All the experimental procedures were approved by the cantonal veterinary office in Zurich (authorization: ZH 182/2018 and ZH 042/2019) and performed in accordance with Swiss animal law and regulations.

2.3 | Peanut allergen sensitization and allergic challenge of mice

C3H mice were actively sensitized by four weekly (days 0, 7, 14, and 21) intraperitoneal injections of 4.2 μg peanut allergen extract (PRICK-TEST Arachis hypogea Lös. Allergopharma, Reinbek, DE) absorbed on 150 μg aluminum hydroxide (Alhydrogel, InvivoGen; San Diego, US-CA) in a total volume of 50 μl with PBS (Figure 2A). Blood was collected via the tail vein for testing of peanut allergen-specific antibodies. Alternatively, BALB/c mice were passively sensitized by adoptive transfer of peanut-specific antiserum. Briefly, cardiac blood was collected from actively sensitized C3H mice on day 49, and 100 μl of a serum pool was intravenously injected into recipient BALB/c mice.

Sensitized mice were challenged intraperitoneally with 1 mg of an in-house preparation of peanut protein extract (PE) contained in 100 μl PBS. The peanut proteins were extracted from Golden Peanut and Tre Nuts (Alpharetta, Georgia, US-GA); the PRICK-TEST and the in-house PE are described in Figures S1–S2. The anaphylaxis was monitored by measuring the core body temperature with a digital Thermalert TH-5 thermometer with RET-3 probe (Physitemp, Huron, US-NJ), typically every 20–30 min for a period of 2 h. Temperature–time curves were integrated and the area under curve (AUC) indicated as a measure for anaphylaxis, the baseline for the AUC curve being the pre-challenge body temperature.

2.4 | PIT with mAbs in a model of systemic peanut anaphylaxis

Actively sensitized mice were treated by intravenous injections of single peanut allergen-specific mAbs provided by Mabylon AG and named "MY" (MY-mAb-1, MY-mAb-2, MY-mAb-3, and MY-mAb-4) or a quadruple mixture of the same mAbs approximately 4 weeks after last sensitization. While single mAbs were tested only on an IgG1 subclass backbone, both IgG1 (MY-4-IgG1) and IgG4 (MY-4-IgG4) mixtures were tested. The utilized doses of mAbs were 0.15 mg to 5 mg per animal (corresponding to 7.5 to 250 mg/kg). In selected experiments, peanut allergen-sensitized mice were sham-treated with IgG1 or IgG4 isotype mAbs. To test if peanut-specific mAbs also act on the inhibitory receptor FcγRIIb, sensitized mice were treated with genetically mutated IgG1 mAbs that were unable to bind Fcγ receptors (MY-4-IgG1$_{\Delta Fc}$). To test the potential of peanut-specific mAbs in preventing peanut-specific anaphylaxis, sensitized and treated mice were challenged intraperitoneally 3 h to 3 days later with peanut protein extract as described above. Passively sensitized BALB/c mice were typically treated the same day of sensitization with peanut-allergen-specific mAbs then challenged 1–3 days later with peanut protein extract. Naïve non-sensitized and untreated mice were used as negative controls.

2.5 | Pharmacokinetics of monoclonal antibodies in mice

The kinetics of anti-peanut mAbs was measured in mouse serum over 4 weeks after intravenous administration of 0.5 mg of MY-4-IgG1 mAbs. The concentration of the human antibody in mouse serum was determined by sandwich ELISA. Plates were coated overnight with 1 μg/ml donkey anti-human IgG (#709–005-149, Jackson ImmunoResearch, West Grove, US-PA) in carbonate buffer at pH 9.6, blocked with 5% Milk in PBST, and serial dilutions of mouse sera were added. Following incubation with peroxidase-conjugated goat anti-human Fcγ-specific IgG (#109–035-098, Jackson ImmunoResearch, 1:4000), the plates were developed with streptavidin-HRP and TMB and analyzed as described (Appendix S1).

Immunogenicity of the therapeutic mAbs were also tested in the same serum samples. The plates were coated with 1 μg/ml MY-4-IgG1 mAbs, blocked with 5% milk PBST, and incubated with serial dilutions of mouse sera. Mouse anti-human IgG1 were detected with biotin-labeled goat anti-mouse IgG1 (#ab97238, Abcam, Cambridge, UK), and the plates developed as described above.

2.6 | Statistics

Statistical analysis was performed with GraphPad Prism 8. Differences between two groups were calculated using non-parametric, two-tailed Mann–Whitney *U* tests. Time–temperature curves in the anaphylaxis model were integrated to calculate the area under the curve (AUC) relative to the baseline temperature. One-way ANOVA with Dunnet's or Tukey correction for multiple comparison or two-way ANOVA were applied to calculate the differences between groups and between treatments. Dose–response curves were analyzed with two-tailed, unpaired t test with Welch's

correction. Significant differences were annotated with exact p values or with asterisks: $p<0.01$: **; $p<0.001$: ***; $p<0.0001$: ****. Means with SD, SEM or geometric means with 95% confidence intervals are indicated.

3 | RESULTS

3.1 | Antibody binding to Ara h 2

Sequential binding experiments using biolayer interferometry (BLI), suggested that a cocktail of four different mAbs can bind a single Ara h 2 molecule simultaneously (Figure S2). The fast k_{on} and slow k_{off} rates, suggested a high dissociation constant KD on nAra h 2 for all mAbs. These results were corroborated by detailed affinity determination in surface plasmon resonance, with all mAbs exhibiting sub-nM KD values (data not shown).

3.2 | Anti-peanut mAbs block IgE binding to Ara h 2 in vitro and prevent degranulation of basophils

One of the hypothesized mechanisms of action of the therapeutic mAbs is the neutralization of Ara h 2, which inhibits IgE crosslinking on the surface of granulocytes thereby blocking degranulation and subsequent allergic reactions (Figure 1A). To determine if the fully human peanut-specific IgG1 mAbs could compete with murine IgE in binding the major peanut allergen Ara h 2, a competitive ELISA was carried out. Single or combined anti-peanut IgG1 mAbs (MY-mAb-1, MY-mAb-2, MY-mAb-3, MY-mAb-4) binding to non-overlapping epitopes were tested, and IgE binding inhibition was indeed observed for all mAbs (Figure 1B). The percentages of inhibition for the individual anti-peanut mAbs were 12.5% for MY-mAb-3, 19.4% for MY-mAb-4, 24.9% for MY-mAb-2, and 41.4% for MY-mAb-1. For mixtures of two mAbs, the inhibition increased to levels between 34.9% (MY-mAb-2 & MY-mAb-3) and 66.7% (MY-mAb-1 and MY-mAb-4). Mixtures of three mAbs further improved the inhibition of IgE binding (48.2% to 64.2%). The strongest inhibition (76.3%) was obtained with MY-4-IgG1, a mixture of all the four IgG1 mAbs (Figure 1B), and the inhibition was dose dependent (Figure 1C). In contrast, the isotype control (targeting irrelevant exogenous target) did not interfere with the binding of Ara h 2 to murine IgE (data not shown). When basophil cells (RBL-2H3) were sensitized with mouse serum and challenged with peanut allergens in the presence of anti-peanut MY-4-IgG1, treatment with MY-4-IgG1 prevented degranulation and leukotriene release (Figure 1D).

3.3 | Antibody response and anaphylactic reactions

In the utilized C3H allergy model (Figure 2A), sensitization produced high titres of anti Ara h 2-specific IgE (Figure 2B) and IgG1 (Figure 2C), with the average reciprocal titres on day 49 being 217 and 8.7×10^5 respectively. Specific IgE and IgG1 were not detectable 7 days after the first sensitizing injection. A systemic challenge at day 49 with peanut protein extract triggered severe hypothermia (Figure 2D) as well as clinical signs of anaphylaxis (orbital tightening, reduced self-grooming, piloerection, hunched posture, reduced mobility and reactions to handling and sounds; results not shown) in sensitized mice. When calculating the temperature–time integral, a highly significant hypothermia was measured in sensitized as compared to naïve mice (Figure 2E).

3.4 | Dose-dependent protection from allergic anaphylaxis in mice treated with a cocktail of four anti-peanut mAbs as IgG1 or IgG4

The therapeutic potential of fully human peanut-specific mAbs was tested in mouse models of allergic anaphylaxis. Sensitized C3H mice were treated by intravenous injection of individual anti-peanut IgG1 mAbs and then 3 days later challenged with peanut protein extract by intraperitoneal injection. Single IgG1 mAbs partially reduced allergic anaphylaxis as compared to untreated mice (not shown). When targeting four peanut-allergen epitopes, immunotherapy with 5 mg (250 mg/kg body weight) MY-4-IgG1 prevented allergic anaphylaxis (Figure 3A). The hypothermic reactions were statistically lower ($p<0.0001$) as compared to the untreated mice, and not different from non-sensitized mice ($p>0.05$). The average AUC for the time–temperature integral was measured and revealed an 89.5% reduction in the treated group (AUC = 74 ± 28) compared to the untreated mice (AUC = 701 ± 75). Non-sensitized and untreated mice were used as negative controls and showed only weak background hypothermia (AUC = 15 ± 3). As illustrated in Figure 3B, a dose–response effect of MY-4-IgG1 on anaphylaxis was observed ($p<0.0001$ by t test and Welch's correction) with mice receiving 0.625 mg (31.25 mg/kg) or more of the anti-peanut IgG1 mAbs being protected from experimental anaphylaxis ($p<0.0001$ by one-way ANOVA with Dunnett's corrections for multiple testing).

In patients that naturally outgrow IgE-mediated food-allergies or receiving conventional non-foods subcutaneous or sublingual AIT, IgG4 antibodies are usually increased. IgG4 possess specific non-inflammatory properties and cannot activate complement. We therefore tested MY-4-IgG4, consisting of four fully human IgG4 mAbs with the same specificity as MY-4-IgG1. At 1.25 mg (62.5 mg/kg) and 0.625 mg (31.25 mg/kg), IgG4 antibodies reduced anaphylaxis by 88.6% ($p<0.001$) and 91.1% ($p<0.001$), respectively, as compared to untreated sensitized mice (Figure 3C). An 80% ($p<0.001$) reduction of anaphylaxis was observed with 0.3125 mg (15.625 mg/kg) MY-4-IgG4, while 0.1563 mg (7.822 mg/kg) reduced anaphylaxis by 61.2% ($p<0.01$). An isotype IgG4 antibody directed against irrelevant target was tested at 1.25 mg (62.5 mg/kg) and did not protect peanut-sensitized mice from anaphylaxis.

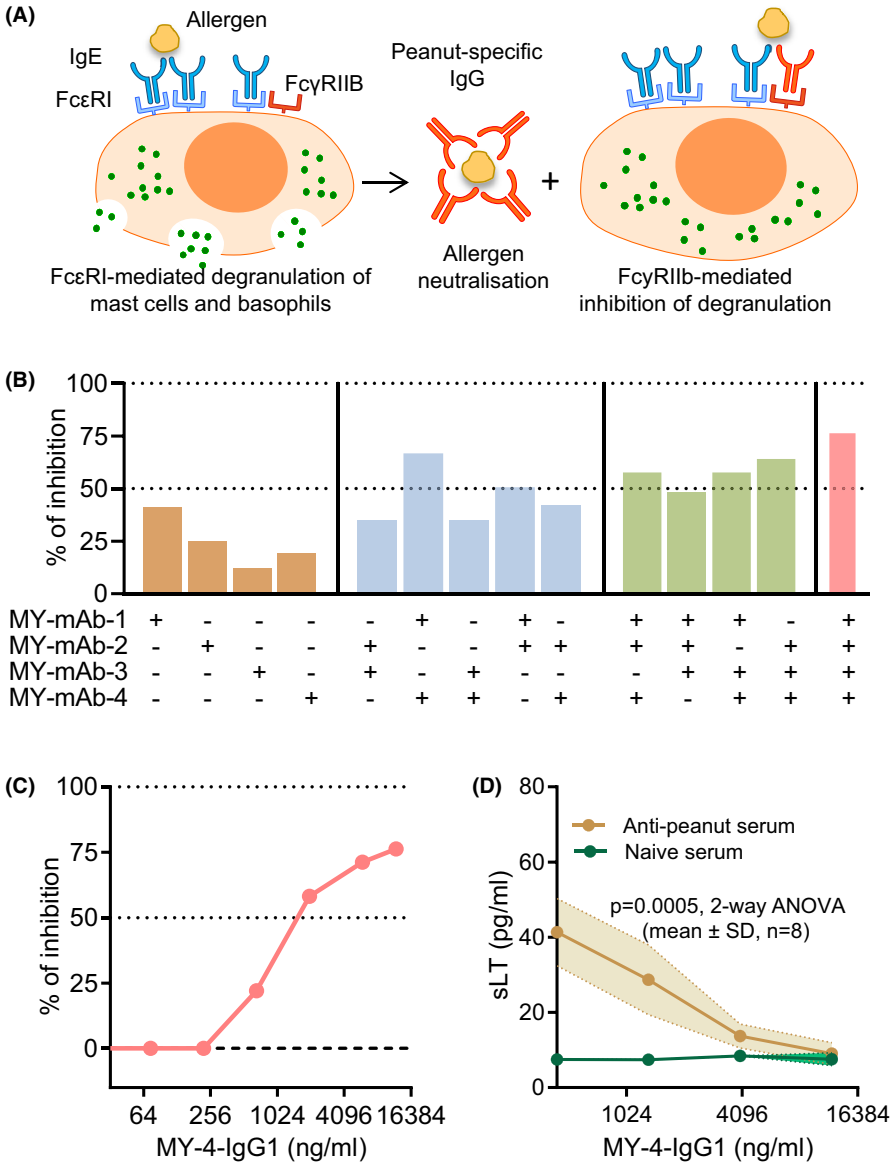


FIGURE 1 Anti-peanut IgG mAbs compete with murine IgE for the binding of Ara h 2 and prevent cellular degranulation. (A) Possible mechanisms by which peanut-specific mAbs can prevent FcεRI-mediated degranulation of effector cells include allergen neutralization, preventing allergen binding to IgE on effector cells, and mAb binding to the FcγRIIb receptor, mediating a receptor-dependent inhibition of effector cells degranulation. (B) Competition of mouse IgE with individual or different combination of 12,000 ng/ml of anti-peanut mAbs (MY-mAb-1, MY-mAb-2, MY-mAb-3, MY-mAb-4) were tested. Orange: one mAb. Light blue: two mAbs. Green: three mAbs. Pink: four mAbs. The percentage of IgE binding inhibition to Ara h 2 is indicated on the y axis. (C) The percentage of binding inhibition of mouse IgE to Ara h 2 in the presence of titrated amount of four human anti-peanut IgG1 (MY-4-IgG1, 0.10–12,000 ng/ml) was evaluated in a sandwich competition ELISA. (D) Leukotriene (sLT) release from RBL-2H3 cells sensitized with anti-peanut or naïve mouse serum and challenged with peanut allergen extract in the presence of increasing doses of MY-4-IgG1.

3.5 | The in vivo half-life of the MY-4-IgG1 mAbs correlates with the development of murine anti-human antibodies

Immunotherapy with MY-4-IgG1 resulted in abrogation of severe allergic reactions from 3 h to 14 days after treatment, while no protection was observed 22 days after immunotherapy (Figure 3D). This result coincided with the decay of mAbs in blood (Figure 3E). One day after the i.v. injection of 0.5 mg (25 mg/kg) MY-4-IgG1, approx. 110 µg/ml human IgG antibodies were detected in the serum. By day 4, a 32.9% reduction was measured (74 µg/ml), and the plasma concentrations remained stable for the following 3 days. On days 14, 20, and 27, the concentrations of human antibodies were reduced to approx. 29, 14, and 7 µg/ml, respectively, suggesting a half-life of approx. 6–7 days. Mice receiving the human anti-peanut mAbs developed murine anti-human antibodies from day 20 onwards (Figure 3F).

3.6 | Immunotherapy with anti-peanut mAbs prevents anaphylaxis in a murine model of passive sensitization

The therapeutic effect of MY-4-IgG1 was also observed in passively sensitized mice (Figure 3G). Passively sensitized mice reacted to the challenge with hypothermia and clinical signs of anaphylaxis within 20–40 min, while immunotherapy with MY-4-IgG1 protected all mice from severe anaphylaxis ($p < 0.001$). PIT with MY-4-IgG1 mAbs (AUC = 79 ± 6) reduced hypothermia by 78.1% as compared to sensitized and challenged mice that did not

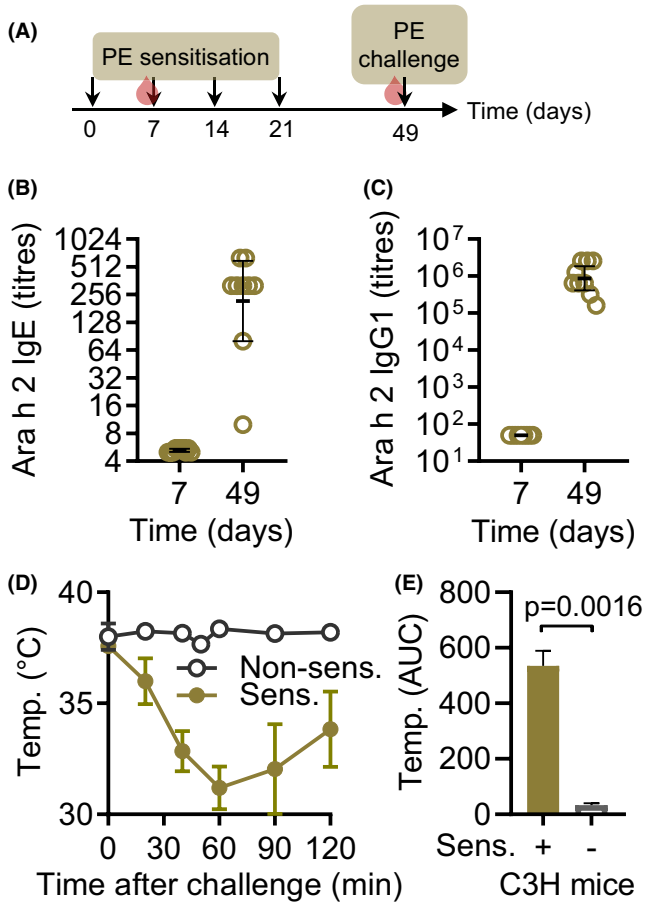


FIGURE 2 Allergen-specific antibodies titres and anaphylaxis in an actively sensitized mouse model for peanut allergy. (A) C3H mice ($n = 8$) were sensitized by four weekly injections of peanut allergen extract (arrow, PE sensitization) absorbed to aluminum hydroxide. Blood was collected on day 7 and 49 and Ara h 2-specific IgE (B) and IgG1 (C) antibody titres were measured by ELISA (statistics were done using Mann–Whitney *U* tests). Four weeks after sensitization, the mice were challenged with peanut protein extract (arrow, PE challenge) and effect on core body temperature was measured every 20–30 min for 120 min. Hypothermia is a hallmark of systemic anaphylaxis. Body temperature is shown as temperature curves (D) and integrated area under the curve (AUC, Mann–Whitney U tests; E). Non-sensitized (Non sens.) mice were used as negative controls. Antibodies titres are represented as geometric mean with 95% CI. Temperature curves are indicated as mean ± SD and AUC as mean ± SEM. ****$p < 0.0001$.

receive PIT (AUC = 360 ± 47), while challenged naïve mice showed no anaphylaxis (AUC = 29 ± 4).

3.7 | Protection from anaphylaxis is mainly mediated through allergen-neutralization and not inhibitory FcγR receptors

To evaluate if the protection from anaphylaxis was also mediated by the activation of the inhibitory receptor FcγRIIb, we compared the Fc-receptor binding capacity (method described in Appendix S1) as well as the therapeutic efficacy of MY-4-IgG1 to that of a mixture of four anti-peanut mutant IgG1 (MY-4-IgG1$_{\Delta Fc}$) unable to bind to Fc receptors on the surface of effector cells. While a complex of MY-4-IgG1 and Ara h 2 could bind FcγRIIb in vitro, the Ara h 2 complex with the mutated isoform IgG1$_{\Delta Fc}$ was unable to bind to FcγRIIb (Figure 4A). Moreover, the treatment of sensitized mice with MY-4-IgG1$_{\Delta Fc}$ was highly effective (One-way ANOVA, $p < 0.0001$) and not inferior ($p < 0.0001$) to treatment with native MY-4-IgG1 with regard to the prevention of anaphylactic hypothermia upon challenge with peanut protein extract (Figure 4B).

4 | DISCUSSION

We tested the immunotherapeutic potential of fully human anti-peanut mAbs in two mouse models of allergic anaphylaxis. The prophylactic administration of a cocktail of four non-competing IgG1 or IgG4 antibodies targeting the major peanut allergen Ara h 2 was highly effective. In general, mixtures of mAbs directed toward different peanut allergen epitopes show a higher therapeutic potential than single mAbs.

The first demonstration of the success of passive immunotherapy dates back to 1890 with the transfer of anti-diphtheria and anti-tetanus antisera from immunized to naïve individuals.[31,32] Later, it was shown that human serum contains IgG antibodies that could neutralize allergens and prevent allergic reactions[33,34] in a dose-dependent manner.[19] Today, PIT with specific antibodies is approved for the treatment of toxins and viral infections.[35–37] Recently, PIT with blocking IgG against the immuno-dominant cat allergen Fel d 1 was proven effective in preventing allergic reactions in mice.[25] PIT with allergen-specific antibodies also increased the IgG/IgE ratio and was effective in the prevention of allergic reactions in a mouse model of pollen allergy.[26] Compared to polyclonal antibodies, mAbs enable higher specificity for the antigen, a standardized potency as well as controlled manufacturing.[38] However, while naturally produced polyclonal antibodies have multiple epitope specificities,[35,39] mAbs are targeting one epitope only.[39] This potential deficit can be overcome by using a cocktail of mAbs binding non-overlapping epitopes.[38]

Ara h 2 is considered to be a very potent activator of sensitized effector cells,[6] and studies reported that neutralization of Ara h 2 with human anti-Ara h 2 monoclonal IgG was effective in inhibiting IgE-FcεRI cross-linking in vitro assays[40] and that PIT with murine anti-Ara h 2[41] or vaccination of mice with Ara h 2 protein[42] could prevent allergic reactions in vivo. Accordingly, we demonstrated that targeting Ara h 2 with fully human mAbs prevented the release of allergic mediators from rat basophilic leukemia cells. We also showed that human mAbs were able to compete with mouse IgE for the binding of Ara h 2 and that the cocktail of four mAbs could prevent anaphylaxis in vivo in two different mouse models in a dose-dependent manner. The strength of the mAbs used in this study, is the fact that they derived directly from allergic individuals, therefore targeting disease-relevant epitopes on Ara h 2. Most importantly, no steric

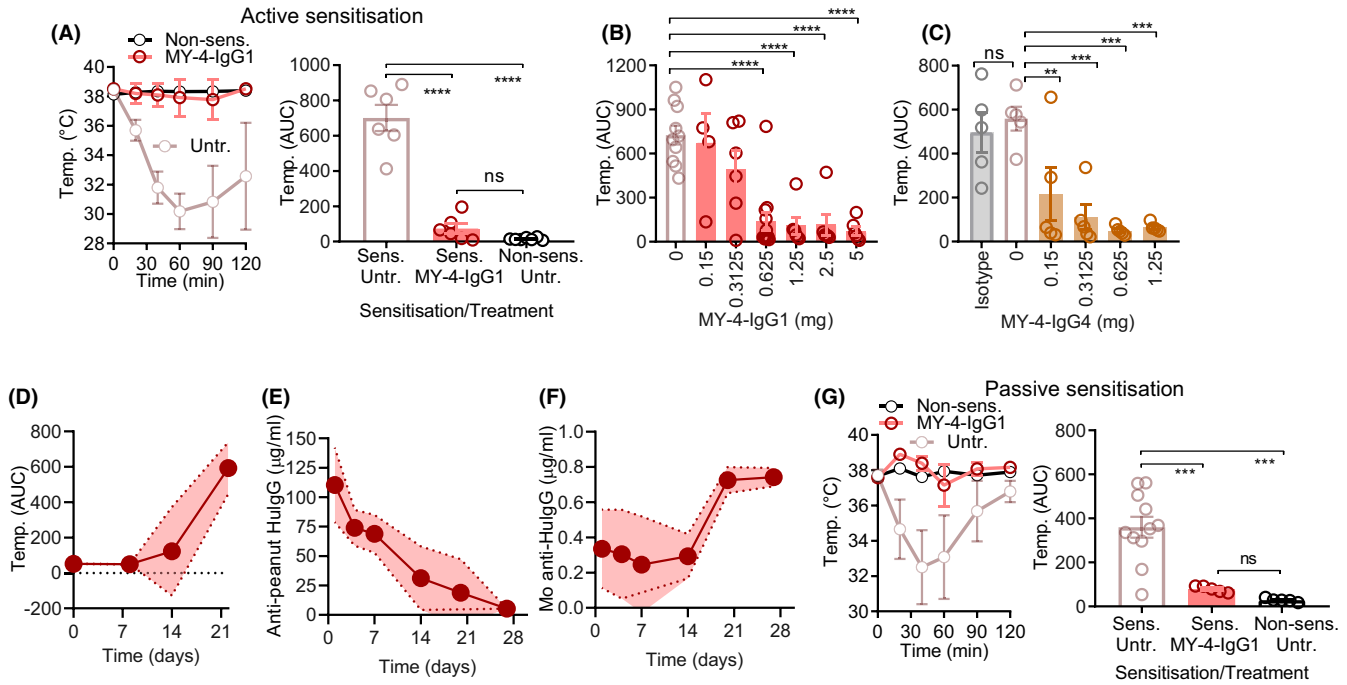


FIGURE 3 In vivo efficacy testing of human-derived anti-peanut mAbs. (A) C3H sensitized ($n = 6$) mice received a cocktail composed of four independent anti-peanut IgG1 mAbs (MY-4-IgG1; 5 mg, 250 mg/kg body weight) and were challenged 1–3 days thereafter. Body temperature was measured after challenge with hypothermia as an indicator for anaphylaxis. Temperature curves (left) and time–temperature integrals (AUC; right) are illustrated (one-way ANOVA with Tukey). Sensitized, but untreated mice, (Untr.; $n = 6$) were used as positive controls. Non-sensitized and untreated (Non sens. Untr.) mice were used as negative controls. (B) Dose–response analysis in C3H sensitized mice ($n = 40$) treated with 0.15–5.0 mg (7.5–250 mg/kg) of the anti-peanut IgG1 cocktail 3 days before the challenge. Body temperature was measured after challenge and shown as AUC (statistical analysis done via one-way ANOVA with Dunnett). Sensitized, but untreated, mice (Untr.; $n = 10$) were used as positive controls. (C) Dose–response analysis in C3H sensitized mice ($n = 25$) treated with different doses of a cocktail of four anti-peanut IgG4 antibodies (MY-4-IgG4; 0.15–1.25 mg, 7.5–62.5 mg/kg). IgG4 isotype control (1.25 mg, 62.5 mg/kg) is directed against an irrelevant target is used as control. Body temperature was measured after challenge (AUC; one-way ANOVA with Dunnett). Sensitized but untreated mice (Untr.; $n = 5$) were used as positive controls. (D) Hypothermia after allergen challenge was measured in C3H sensitized mice ($n = 4$) injected with 2.7 mg (135 mg/kg) MY-4-IgG1 3 hours (day 0) and 8, 14, and 22 days before challenge. The concentrations of human anti-peanut IgG (E) and of mouse anti-human IgG1 (F) were measured in C3H mouse serum by ELISA over a period of 4 weeks after the administration of the MY-4-IgG1 antibodies (0.5 mg, 25 mg/kg). (G) BALB/c passively sensitized mice ($n = 5$) were administered with the cocktail of four antibodies (MY-4-IgG1; 2.5 mg, 125 mg/kg) and challenged on the following day. Body temperature (left) and AUC (right) are shown (one-way ANOVA with Tukey). Sensitized, but untreated, mice (Untr.; $n = 11$) were used as positive controls. Non-sensitized (Non sens.) mice were used as negative controls. Temperature curves and integrated AUC are represented as mean ± SD and mean ± SEM, respectively. Antibodies concentrations are expressed as mean and error with 95% CI. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. ns, non-significant.

hindrance between the mAbs was observed and all antibodies were able to bind to a single Ara h 2 molecule simultaneously. This explains the strong additive effect when using a cocktail, compared to single mAbs. The fact that anti-peanut mAbs derived from peanut-allergic patients compete with mouse IgE for the binding of Ara h 2 and prevent peanut-allergic reactions in the mouse models suggest that the epitope coverage is at least partially conserved between mice and humans. Although humanized mice are often considered valuable models that resemble human allergy,[43,44] our mouse model was suitable for testing the therapeutic efficacy of anti-peanut mAbs, overcoming potential pitfalls of humanized mice.[45,46]

Albeit human IgG have the capability to bind murine Fc receptors with similar affinities,[47] we showed that the therapeutic effect of anti-peanut mAbs was primarily mediated through allergen neutralization and not by the binding of inhibitory FcγRIIb, as previously suggested.[40] Indeed, treatment with genetically mutant mAbs unable to bind Fc receptors was not inferior to wild-type IgG1 mAbs in preventing allergic anaphylaxis in mice. However, contrasting results exist[41] indicating that beside allergen neutralization, the low affinity FcγRIIb might have an inhibitory effect, probably at very high concentrations of mAbs.[48]

Decay of the therapeutic effect was observed in mice 22 day after PIT, most likely due to the species mismatch and the emergence of anti-human antibodies, which caused faster clearance of administered mAbs.[49] Additionally, these anti-drug antibodies have the potential to be neutralizing, also negatively influencing the therapeutic efficacy of the cocktail mAbs. In clinical trials, however, the half-life of IgG following PIT were 3–4 weeks,[50,51] suggesting that the therapeutic efficacy of anti-peanut antibodies in allergic patients could last for at least 2 months. It is nevertheless known that

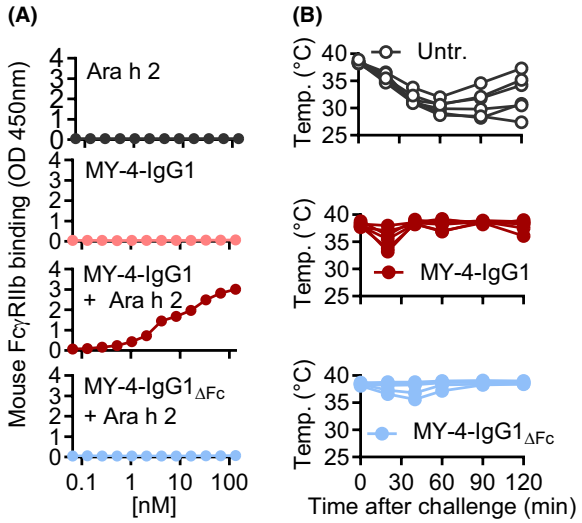


FIGURE 4 Therapeutic efficacy of Fc-mutated IgG mAbs. (A) ELISA for the detection of mouse Fcγ receptor binding by IgG1 and Fc-mut IgG1 ($IgG1_{\Delta Fc}$) mAbs complexed with Ara h 2 (133 nM mAbs +133 nM Ara h 2). Uncomplexed IgG1 and Ara h 2 were used as negative control. (B) C3H sensitized mice received intravenous injections of a mixture of four anti-peanut IgG1 (MY-4-IgG1; $n = 6$) or same IgG1 without Fc-receptor-binding motive (MY-4-$IgG1_{\Delta Fc}$; $n = 6$) mAbs (1.25 mg, 62.5 mg/kg) prior to i.p. allergen challenge. Body temperatures upon challenge are illustrated. Sensitized, but untreated, mice (Untr.; $n = 6$) were used as positive controls.

anti-drug antibodies can be triggered even by fully human antibodies,[52] but avoiding impurities in the formulation process, an appropriate evaluation of mAbs dosage as well as the number of injections are fundamental parameters that can be controlled in order to reduce antibodies immunogenicity.[52] To date, more than 70 antibody-based treatments are on the market, and many more are in different stages of clinical trials.[52,53]

The only approved treatment for peanut allergy is oral immunotherapy (OIT), during which small amounts of allergens are administered orally in order to induce peanut tolerance[14] including reduced allergen-specific IgE and increased allergen-specific IgG levels, especially IgG4.[15,54] IgG4 has become an important focus in allergy research,[55] already proven effective in blocking the interaction between aeroallergens and FcεRI-bound IgE,[56] and associated with recovery from milk allergy and tolerance to egg proteins.[57,58] Hence, although PIT in mice using the IgG1 subclass protected against anaphylaxis, future therapeutic approaches will most likely be using the IgG4 subclass for treatment of allergy. In the current study, a single PIT with IgG4 was non-inferior to therapy with IgG1 in mice. It should be noted that IgG4 has certain characteristics that confer stronger anti-inflammatory properties compared to other IgG subclasses, leading to immune tolerance.[59–64] IgG4 are unable to fix the complement system, avoiding its uncontrolled activation with an exacerbation of local or systemic inflammation causing inappropriate tissue damage.[65,66] Hence, an antibody-based therapy to treat allergies will most likely be based on the IgG4 subclass.

Not all patients undergoing peanut OIT develop tolerance,[14] and 15%–20% of the patients have OIT-triggered persistent gastrointestinal symptoms with an elevated risk of developing adverse reactions including anaphylaxis.[67,68] Given these concerns regarding the safety of OIT or other AITs,[69] prophylactic therapeutic administration of peanut-specific IgG antibodies can be considered a promising approach in the treatment of peanut allergy.

In conclusion, we demonstrated that fully human anti-peanut mAbs, cloned from B cells of peanut-allergic patients, are functional, can bind and neutralize peanut allergens, and be effective in the protection from allergic anaphylaxis in vivo. In the applied mouse model, therapeutic effect were achieved at doses of less than 10 mg/kg, corresponding to 600–800 mg in humans. This is similar to clinical doses used in trials with anti-Bet v 1 mAbs[29] and approved for many checkpoint or growth factor inhibitors. Nonetheless, due to the species differences, it is expected that effective doses in human are on average 12 times lower,[70] which will have both safety and health economic impact for a chronic treatment. Finally, even if peanut allergy is driven by multiple allergenic proteins, we showed that targeting the dominant allergen with mAbs abrogates anaphylaxis in preclinical mouse models of peanut allergy. These findings may pave the way for mAb PIT as a new clinical option for patients with difficult or untreatable allergies.

AUTHOR CONTRIBUTIONS

MP performed experimental design and analysis, data analysis, and wrote the first version of the manuscript. VH performed experimental analysis performed data analysis. YW-M performed experimental analysis. AK, BBW, NW, DB, NP, and TS provided material and/or helped design the experiments and analysis. TK provided material and funding. PJ performed experimental design, data analysis, wrote the manuscript with MP, and had the general project responsibility. All authors read, revised, and approved the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to Agathe Duda, Chiara Arena, and Simon Christiaanse for experimental support.

FUNDING INFORMATION

The project received funds from Innosuisse – Swiss Innovations Agency (25866.2 PFLS-LS). Marta Paolucci received a Swiss Government Excellence Scholarships for Foreign Scholars and Artists (2019.0338). Further support for overhead was received from the University of Zurich and from the Truus und Gerrit van Riemsdijk Stiftung (Vaduz, Lichtenstein).

CONFLICT OF INTEREST

Natascha Wuillemin, Dimitri Bieli, Niccolò Pengo, and Tiziana Sonati are employees of Mabylon AG, which develops immunotherapies against peanut allergy. Thomas Kündig is scientific advisor to Mabylon AG. All other authors declare no conflict of interest.

ORCID

Marta Paolucci https://orcid.org/0000-0001-5371-0514
Niccolò Pengo https://orcid.org/0000-0002-9249-4786
Pål Johansen https://orcid.org/0000-0002-5055-6299

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol*. 2018;141(1):41-58.
2. Loh W, Tang MLK. The epidemiology of food allergy in the global context. *Int J Environ Res Public Health*. 2018;15(9):2043.
3. Lieberman JA, Gupta RS, Knibb RC, et al. The global burden of illness of peanut allergy: a comprehensive literature review. *Allergy*. 2021;76(5):1367-1384.
4. Kanagaratham C, El Ansari YS, Lewis OL, Oettgen HC. IgE and IgG antibodies as regulators of mast cell and basophil functions in food allergy. *Front Immunol*. 2020;11:603050.
5. Üzülmez Ö, Kalic T, Mayr V, et al. The Major Peanut allergen Ara h 2 produced in Nicotiana benthamiana contains Hydroxyprolines and is a viable alternative to the *E. Coli* product in allergy diagnosis. *Front Plant Sci*. 2021;12:723363. doi:10.3389/fpls.2021.723363
6. Hemmings O, Du Toit G, Radulovic S, Lack G, Santos AF. Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6. *J Allergy Clin Immunol*. 2020;146(3):621-630.
7. Zhuang Y, Dreskin SC. Redefining the major peanut allergens. *Immunol Res*. 2013;55(1–3):125-134.
8. Paolucci M, Wuillemin N, Köhli A, et al. Multivariate allergen-specific analysis and profiling of serum antibodies from patients with peanut allergy. *Clin Exp Allergy*. 2022. doi:10.1111/cea.14262 Epub ahead of print.
9. Koppelman SJ, Wensing M, Ertmann M, Knulst AC, Knol EF. Relevance of Ara h1, Ara h2 and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen. *Clin Exp Allergy*. 2004;34(4):583-590.
10. Cummings AJ, Knibb RC, King RM, Lucas JS. The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: a review. *Allergy*. 2010;65(8):933-945.
11. Maris I, Dölle-Bierke S, Renaudin JM, et al. Peanut-induced anaphylaxis in children and adolescents: data from the European anaphylaxis registry. *Allergy*. 2021;76(5):1517-1527.
12. Anagnostou K, Clark A. Peanut immunotherapy. *Clin Transl Allergy*. 2014;4:30.
13. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol*. 1992;90(2):256-262.
14. Mullard A. FDA approves first peanut allergy drug. *Nat Rev Drug Discov*. 2020;19(3):156.
15. Sugimoto M, Kamemura N, Nagao M, et al. Differential response in allergen-specific IgE, IgGs, and IgA levels for predicting outcome of oral immunotherapy. *Pediatr Allergy Immunol*. 2016;27(3):276-282.
16. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2011;127(1):18-27. quiz 28–19.
17. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*. 2014;133(3):621-631.
18. Vickery BP, Lin J, Kulis M, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *J Allergy Clin Immunol*. 2013;131(1):128-134.
19. Turner P, Patel N, Campbell D, et al. Increase in IgG4 following Peanut Oral immunotherapy is primarily limited to a single specific epitope against Ara h 2. *J Allergy Clin Immunol*. 2020;145(2 Supplement):AB339.
20. Vickery BP, Lin J, Kulis M, et al. Peanut Oral immunotherapy (OIT) induces epitope-specific isotype shift from IgE to IgG4. *J Allergy Clin Immunol*. 2010;125(2 Supplement 1):AB20.
21. Santos AF, James LK, Bahnson HT, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol*. 2015;135(5):1249-1256.
22. Hingley S, Du Toit G, Roberts G, et al. Peanut specific IgG4 and its association with Peanut allergy. *J Allergy Clin Immunol*. 2006;117(2 Supplement):S33.
23. Chinthrajah RS, Cao S, Dunham T, et al. Oral immunotherapy for peanut allergy: the pro argument. *World Allergy Organ J*. 2020;13(8):100455.
24. Flicker S, Valenta R. Renaissance of the blocking antibody concept in type I allergy. *Int Arch Allergy Immunol*. 2003;132(1):13-24.
25. Orengo JM, Radin AR, Kamat V, et al. Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement. *Nat Commun*. 2018;9(1):1421.
26. Flicker S, Linhart B, Wild C, Wiedermann U, Valenta R. Passive immunization with allergen-specific IgG antibodies for treatment and prevention of allergy. *Immunobiology*. 2013;218(6):884-891.
27. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and fc gamma RIIb cross-linking. *J Clin Invest*. 2006;116(3):833-841.
28. Uermösi C, Beerli RR, Bauer M, et al. Mechanisms of allergen-specific desensitization. *J Allergy Clin Immunol*. 2010;126(2):375-383.
29. Gevaert P, De Craemer J, De Ruyck N, et al. Novel antibody cocktail targeting bet v 1 rapidly and sustainably treats birch allergy symptoms in a phase 1 study. *J Allergy Clin Immunol*. 2022;149(1):189-199.
30. Atanasio A, Franklin MC, Kamat V, et al. Targeting immunodominant bet v 1 epitopes with monoclonal antibodies prevents the birch allergic response. *J Allergy Clin Immunol*. 2022;149(1):200-211.
31. Behring K. On the development of immunity to diphtheria and tetanus in animals. *Dtsch Med Wochenschr*. 1965;90(49):2183.
32. Chow SK, Casadevall A. Monoclonal antibodies and toxins—a perspective on function and isotype. *Toxins (Basel)*. 2012;4(6):430-454.
33. Loveless MH. Immunological studies of pollinosis: I. the presence of two antibodies related to the same pollen-antigen in the serum of treated hay-fever patients. *J Immunol*. 1940;38(1):25-50.
34. Loveless MH. Immunological studies of pollinosis IV. The relationship between thermostable antibody in the circulation and clinical immunity. *J Immunol*. 1943;47(2):165-180.
35. Nowakowski A, Wang C, Powers DB, et al. Potent neutralization of botulinum neurotoxin by recombinant oligoclonal antibody. *Proc Natl Acad Sci U S A*. 2002;99(17):11346-11350.
36. Cao Y, Su B, Guo X, et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent Patients' B cells. *Cell*. 2020;182(1):73-84.
37. Parren PW, Geisbert TW, Maruyama T, Jahrling PB, Burton DR. Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. *J Virol*. 2002;76(12):6408-6412.
38. Diamant E, Torgeman A, Ozeri E, Zichel R. Monoclonal antibody combinations that present synergistic neutralizing activity: a platform for next-generation anti-toxin drugs. *Toxins (Basel)*. 2015;7(6):1854-1881.
39. Slifka MK, Amanna IJ. 8 - Passive immunization. In: Plotkin SA, Orenstein WA, Offit PA, Edwards KM, eds. *Plotkin's Vaccines*. 7th ed. Elsevier; 2018:84.e10-95.e10.
40. Liao S, Patil SU, Shreffler WG, Dreskin SC, Chen X. Human monoclonal antibodies to Ara h 2 inhibit allergen-induced, IgE-mediated cell activation. *Clin Exp Allergy*. 2019;49(8):1154-1157. doi:10.1016/B978-0-323-35761-6.00008-0
41. Storni F, Cabral-Miranda G, Roesti E, et al. A single monoclonal antibody against the Peanut allergen Ara h 2 protects against

systemic and local Peanut allergy. *Int Arch Allergy Immunol*. 2020;181(5):334-341.
42. Storni F, Zeltins A, Balke I, et al. Vaccine against peanut allergy based on engineered virus-like particles displaying single major peanut allergens. *J Allergy Clin Immunol*. 2020;145(4):1240-1253.
43. Bryce PJ, Falahati R, Kenney LL, et al. Humanized mouse model of mast cell-mediated passive cutaneous anaphylaxis and passive systemic anaphylaxis. *J Allergy Clin Immunol*. 2016;138(3):769-779.
44. Burton OT, Stranks AJ, Tamayo JM, Koleoglou KJ, Schwartz LB, Oettgen HC. A humanized mouse model of anaphylactic peanut allergy. *J Allergy Clin Immunol*. 2017;139(1):314-322.
45. Laudanski K, Stentz M, DiMeglio M, Furey W, Steinberg T, Patel A. Potential pitfalls of the humanized mice in modeling sepsis. *Int J Inflam*. 2018;2018:6563454.
46. Villaudy J, Schotte R, Legrand N, Spits H. Critical assessment of human antibody generation in humanized mouse models. *J Immunol Methods*. 2014;410:18-27.
47. Dekkers G, Bentlage AEH, Stegmann TC, et al. Affinity of human IgG subclasses to mouse fc gamma receptors. *MAbs*. 2017;9(5):767-773.
48. Bruhns P, Iannascoli B, England P, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood*. 2009;113(16):3716-3725.
49. Loeffler DA, Smith LM, Klaver AC, et al. Development of antihuman IgG antibodies and hematologic deficits but not clinical abnormalities in C57BL/6 mice after repeated administration of human intravenous immunoglobulin. *Comp Med*. 2012;62(1):31-36.
50. Keizer RJ, Huitema AD, Schellens JH, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. *Clin Pharmacokinet*. 2010;49(8):493-507.
51. Kamal MA, Dingman R, Wang CQ, et al. REGN1908-1909 monoclonal antibodies block Fel d 1 in cat allergic subjects: translational pharmacokinetics and pharmacodynamics. *Clin Transl Sci*. 2021;14(6):2440-2449.
52. Vaisman-Mentesh A, Gutierrez-Gonzalez M, DeKosky BJ, Wine Y. The molecular mechanisms that underlie the immune biology of anti-drug antibody formation following treatment with monoclonal antibodies. *Front Immunol*. 2020;11:1951.
53. Grilo AL, Mantalaris A. The increasingly human and profitable monoclonal antibody market. *Trends Biotechnol*. 2019;37(1):9-16.
54. Schoos AM, Bullens D, Chawes BL, et al. Immunological outcomes of allergen-specific immunotherapy in food allergy. *Front Immunol*. 2020;11:568598.
55. Wisniewski J, Agrawal R, Woodfolk JA. Mechanisms of tolerance induction in allergic disease: integrating current and emerging concepts. *Clin Exp Allergy*. 2013;43(2):164-176.
56. Burton OT, Logsdon SL, Zhou JS, et al. Oral immunotherapy induces IgG antibodies that act through FcγRIIb to suppress IgE-mediated hypersensitivity. *J Allergy Clin Immunol*. 2014;134(6):1310-1317.
57. Savilahti EM, Rantanen V, Lin JS, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *J Allergy Clin Immunol*. 2010;125(6):1315-1321.
58. James JM, Sampson HA. Immunologic changes associated with the development of tolerance in children with cow milk allergy. *J Pediatr*. 1992;121(3):371-377.
59. James LK, Till SJ. Potential mechanisms for IgG4 inhibition of immediate hypersensitivity reactions. *Curr Allergy Asthma Rep*. 2016;16(3):23.
60. Lilienthal GM, Rahmöller J, Petry J, Bartsch YC, Leliavski A, Ehlers M. Potential of murine IgG1 and human IgG4 to inhibit the classical complement and Fcγ receptor activation pathways. *Front Immunol*. 2018;9:958.
61. Bianchini R, Karagiannis SN, Jordakieva G, Jensen-Jarolim E. The role of IgG4 in the fine tuning of tolerance in IgE-mediated allergy and cancer. *Int J Mol Sci*. 2020;21(14):5017.
62. Eckl-Dorna J, Villazala-Merino S, Linhart B, et al. Allergen-specific antibodies regulate secondary allergen-specific immune responses. *Front Immunol*. 2018;9:3131.
63. Shamji MH, Wilcock LK, Wachholz PA, et al. The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. *J Immunol Methods*. 2006;317(1–2):71-79.
64. Flicker S, Gadermaier E, Madritsch C, Valenta R. Passive immunization with allergen-specific antibodies. *Curr Top Microbiol Immunol*. 2011;352:141-159.
65. Morgan BP, Harris CL. Complement, a target for therapy in inflammatory and degenerative diseases. *Nat Rev Drug Discov*. 2015;14(12):857-877.
66. Goldberg BS, Ackerman ME. Antibody-mediated complement activation in pathology and protection. *Immunol Cell Biol*. 2020;98(4):305-317.
67. Nadeau KC, Schneider LC, Hoyte L, Borras I, Umetsu DT. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. *J Allergy Clin Immunol*. 2011;127(6):1622-1624.
68. MacGinnitie AJ, Rachid R, Gragg H, et al. Omalizumab facilitates rapid oral desensitization for peanut allergy. *J Allergy Clin Immunol*. 2017;139(3):873-881.
69. Sun J, Hui X, Ying W, Liu D, Wang X. Efficacy of allergen-specific immunotherapy for peanut allergy: a meta-analysis of randomized controlled trials. *Allergy Asthma Proc*. 2014;35(2):171-177.
70. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*. 2016;7(2):27-31.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Paolucci M, Wuillemin N, Homère V, et al. Targeting Ara h 2 with human-derived monoclonal antibodies prevents peanut-induced anaphylaxis in mice. *Allergy*. 2023;78:1605-1614. doi:10.1111/all.15659